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NEURAL PROSTHESIS PROGRAM.

Quarterly Progress to: National Institutes of Health
Contract Monitor William Heetderks, Ph.D.
Research Contract: Surface Modification for Biocompatibility
Contract Number: NS 5-2322 (12)
Principal Investigators: David C. Martin, and K. Sue O'Shea
Date: 31 January, 1998

The Martin Laboratory:

Mike Johnson, Ph.D. candidate in David's laboratory has recently worked for six weeks in San Diego, CA, with Dr. Joseph Cappello to synthesize additional SLPF to continue our studies. He has coated a number of probes for the St. Louis group with mixed results. We are in the process of arranging a visit for that group to Ann Arbor to test the *in vitro* response of neurons and glia to SLPF and SLPL coated substrates using cells isolated from fish, prior to doing additional probe implants. The problems have been with coating -- perforations in the sieve electrodes were often occluded by polymer. In addition, it is not known if cells from the lungfish will respond to laminin (or the IKVAV peptide) or to fibronectin, so this and the ideal size of the sieve will be directly determined. Dr. David Anderson is arranging the meeting.

The O'Shea Laboratory:

Is preparing the results of experiments producing forced-choice patterning of substrates for publication. We have acquired all the images of cell response on the patterned substrates, and Dr. Libby Louie, who is responsible for much of this work, will analyze distance migrated, neurite outgrowth, spreading, etc., using NIH Image. One of the results of Dr. Louie's poster at the Society for Neuroscience meetings last year is a tremendous interest by the neuroscience audience in the reliable production of patterned substrates. Much of this work was done by Dr. Louie at the Cornell Nanofabrication Laboratory, and we are investigating ways of bringing the technology to Ann Arbor.

We have also recently upgraded our Fluovert inverted microscope to allow us to carry out requisite studies of cell-substrate interaction using interference reflection microscopy, by purchasing a new Chroma cube. The degree of cell membrane - substrate interaction will be a particularly important measure of cell response to the polymer substrates.

Also on the biological side, we have this week made very exciting progress in our ability to visualize the implanted electrodes in the Guinea pig cortex. Chris Edwards,

Cell Biology Laboratory manager, and Peter Finger, from the Kresge Hearing Research Institute group, have embedded brain containing electrodes in LR White resin, rather than Spurr's (which we had previously employed). The LR White block is ground until an electrode is visible, then stained with 0.1 % aqueous acridine orange for 1.5h, and visualized in our Biorad laser confocal microscope as we have done previously. Figure One illustrates the tissue morphology using this technique. The nuclei are intensely fluorescent and have very prominent nucleoli. Figure One A,B illustrate low and high magnification views of cortex; C illustrates a region composed of fiber tracts and intensely labeled glial cell nuclei.

We are particularly excited about the ability to use LR White because as demonstrated by post-embedding staining with AO (we had previously stained the tissue *en bloc* with AO prior to embedding), we should be able to use this technique to localize antibodies specific for glial cells, for neuronal processes, and very importantly to identify the sheath of flattened cells that often surrounds the probe. For the Martin-O'Shea group, this will be particularly powerful, as it will also allow us to employ the antibody to the polymer itself, so that we can directly determine the nature of the polymer coating *in situ*. Does the polymer coating remain intact? Do cells interact directly with the polymer *in vivo*? With the existing specimen we anticipate being able to do immunohistochemical localization of markers for fibroblasts, glial and neuronal cells very rapidly.

PLANS for the next quarter:

David Martin continues on sabbatical in Germany, where a portion of his research involves the development and analysis of polymer coatings which are conductive. We are hopeful that these will form a powerful adjunct to be used at the functional regions of the electrode, in combination with adhesive polymer coatings. Mike Johnson in David's lab continues to assess the physical properties of the coatings as a portion of his Ph.D. research, and to coat probes as requested by other researchers.

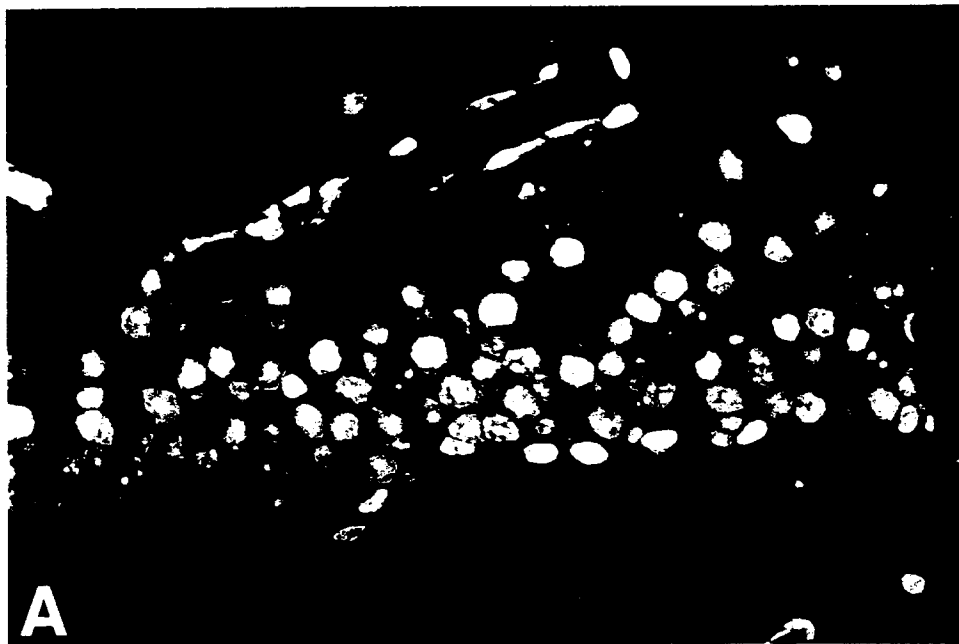
The O'Shea lab continues the analysis of cell behavior on patterned substrates. The requisite images have been collected for analysis using NIH Image which will be carried out by Dr. Louie. We will also continue working to obtain high quality interference reflection images to assess cell-substrate behavior.

Detailed Procedures:

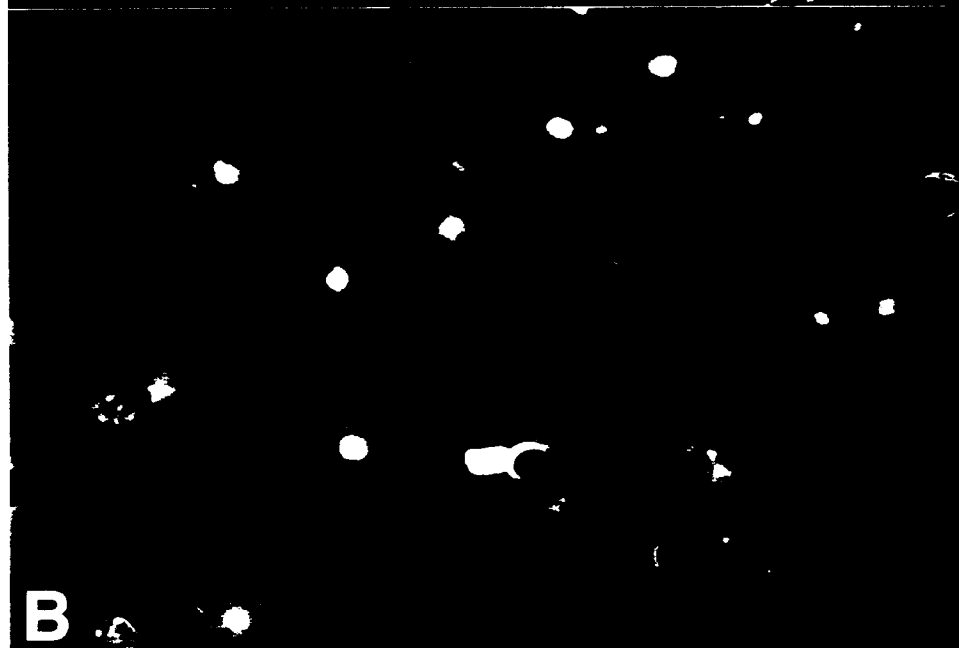
A fixed block of Guinea pig cortex was dehydrated, and placed directly into LR White resin (Hard). Following several changes of resin over a several week period, the tissue was embedded and polymerized at 50 °C for 48h. The resulting block was bisected using the EXAKT band saw and one piece glued to an acrylic microscope slide. The band saw was again employed to cut a 1 mm section which was then polished, giving a final slide with approximately 500 micrometers tissue. The slide was lightly etched in 2% hydrogen peroxide for 20 minutes, rinsed in tap water, then stained in an aqueous solution of 1% acridine orange for 1.5 hours at room temperature. The slide was then washed in running water, air dried, and examined using the Bio-Rad MRC 600 laser confocal microscope using rhodamine optics. The AO staining was apparent approximately 20 micrometers deep in the specimen, and unlike Spurr's, the specimen did not deform over time in the beam.

Micrographs A and B illustrate AO staining of nuclei (including neuronal and endothelial) at low and high magnification. C illustrates scattered glial nuclei in a white matter region.

Figure Two illustrates a high magnification view of the probe tip.



A



B



C

